

## *Trichomonas vaginalis* IN GRAM-STAINED SMEARS\*†

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The case in favour of using a stained smear technique for the recognition of *Trichomonas vaginalis* in clinic practice was admirably put by Liston and Lees (1940) and reiterated by Harrison (1959). The stains advocated by these authors were Leishman's and Löffler's alkaline methylene blue. Kean and Day (1954) and Oller (1965) favoured the Papanicolaou method, but this does not lend itself to routine clinic use. Whittington (1957) considered the stained smear technique to be unreliable.

In late 1964, when examining vaginal smears stained by Gram's method, the author recognized undoubted trichomonads, the nucleus, flagellae, and axostyle being clearly identifiable; thereupon several methods of preparing stained specimens were investigated. The clearest results were finally obtained from smears prepared by dipping a glass rod into a pool of exudate at the posterior fornix, gently rolling the rod on the slide, air fixing, and staining by Gram's method, using 1 per cent. safranin counterstain. This method had been tested by Fowler (1953). Aids to recognition of the parasite were the associated flora, small Gram-negative diplococci, and the long Gram-negative or occasionally positive *Leptothrix*, mentioned as a clue by Papanicolaou and Wolinska (1955). Their presence is not always indicative of trichomonal infestation as has been shown by Carvalho, Kramer, and Kay (1965); trichomonads may be found with all grades of vaginal smear (Schröder, 1921) with or without Döderlein's bacilli and pus.

The trichomonad is Gram-negative, of variable shape, and distinguished by the eccentric lenticular nucleus and vacuolated or foamy cytoplasm. The flagellae are often visible. Epithelial cells are unlikely to be confused with the organism, but a large

stripped nucleus with a prominent nucleolus may be so (Fowler, 1953).

Fig. 1 shows the amount of detail discernible by this method, here enhanced by the use of a green filter.

Fig. 2 shows the trichomonad centrally, surrounded by polymorphonuclear leucocytes and pseudomycelia of *Candida*.

Fig. 3 demonstrates the distortion of cell-shape often seen. Identification is based on observation of the eccentric lenticular nucleus, the foamy cytoplasm, and, in the central organism, the flagellae.

With increasing experience it was found that trichomonads could be recognized more quickly and more frequently by the stained technique than by examining wet preparations. Unfortunately, the time available in busy clinic sessions did not allow routine checking of each stained smear by examination of a wet specimen; accordingly, comparison was made with the results of simultaneous culture using the medium described by Oller (1965). Cultures were read in the Public Health Laboratory, Bristol.

### Material and Method

961 women were examined: 487 consecutive female patients from the Department of Venereal Diseases, United Bristol Hospitals, and 474 unselected cases from an antenatal diagnostic clinic; 45 cases were later excluded because certain data had not been recorded, leaving a total of 916 cases for assessment of results.

All these cases underwent examination by both stained smear and culture, the time allowed for scanning each slide being limited to 2 minutes.

### Results

Culture results were positive in 249 and smear results in 209 of the 916 cases tested, the percentage incidence by culture being 27 per cent. 45 of the specimens giving negative culture results were

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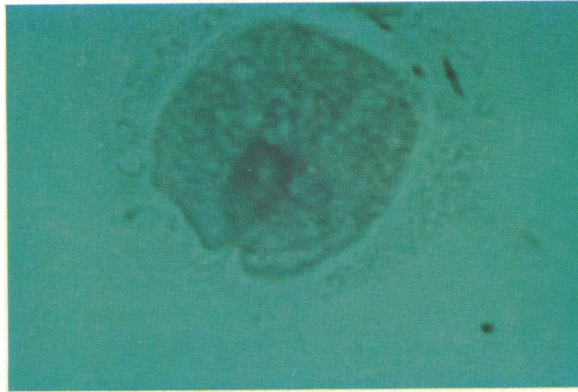


FIG. 1.—*Trichomonas vaginalis* vaginal smear. Gram stain.  $\times 900$ .

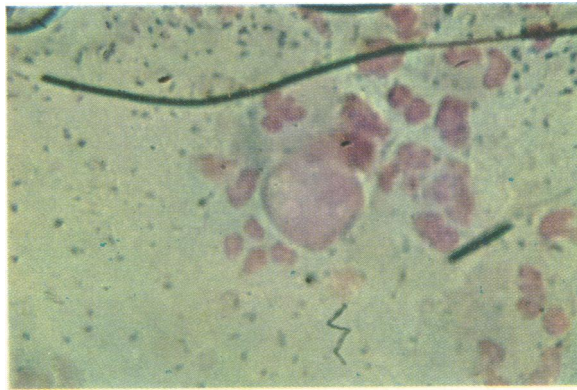


FIG. 2.—Vaginal smear. Gram stain. Mixed flora. *Trichomonas vaginalis*, pseudomycelia of *Candida*, and pus cells.  $\times 850$ .

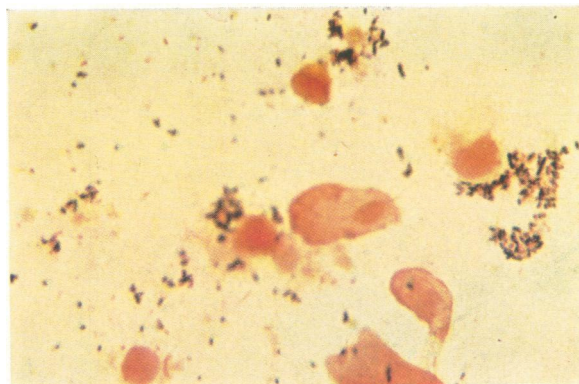


FIG. 3.—Vaginal smear. Gram stain. *Trichomonas vaginalis* and Döderlein's bacilli.  $\times 850$ .

positive in stained smears and the incidence of positive results by culture, slide, or both was 32 per cent. These findings are tabulated below:

	Stain		
	+ ve	- ve	Total
Culture			
+ ve	164	85	249
- ve	45	622	667
Total	209	707	916

Since the employment of a new slide technique raises the possibility of failure of recognition or of false identification of the parasite, the above findings have been analysed by the  $\chi^2$  method; this has shown that the close correspondences between slide and culture findings with both positive and negative results are highly unlikely to have occurred by chance.

$$\chi^2 = 359.8 ; P = < 1:1000.$$

### Summary

A method for the rapid identification of *Trichomonas vaginalis* using Gram-stained smears of vaginal discharge is described. The results have been compared with those obtained by using cultures and a close correspondence between the two methods has been demonstrated.

I should like to thank the Director of the Public Health Laboratory Service, Bristol, for arranging to read the cultures, Dr A. E. Tinkler for his advice and criticism, Mr W. Howell, F.I.T.V., for preparing and checking the slides, Mr A. Todd of Microinstruments (Oxford) for Fig. 1, and the Board of the Bristol Royal Infirmary for a grant towards the cost of publication.

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### Le trichomonas vaginalis dans les frottis colorés avec la solution de Gram

#### RÉSUMÉ

Une méthode rapide pour l'identification du *Trichomonas vaginalis* en se servant des frottis des pertes vaginales colorés par la méthode de Gram est décrite. Les résultats ont été comparés à ceux obtenus des cultures, et une étroite relation entre les deux méthodes a été démontrée.